As taught on p. 9, ll. 27-28, it is known that the gp41 portion of the trimer is sufficient for trimer formation. Furthermore, it is known by those skilled in the art that that gp41 hydrophobic helix is important for trimer formation. Chan and Kim, Cell, Vol. 93, 681–684, May 29, 1998 (copy enclosed). Thus, it is well recognized that less that the entire gp41 protein is required for trimer formation. Indeed, Example 2 of the specification describes an embodiment comprising the first 129 amino acids of the gp41 N-terminal domain, which fragment lacks the transmembrane domain, as described on p. 19. (New claims 30 and 31 are drawn to such embodiments.) Accordingly, it would be a routine matter for one skilled in the art to make and use the claimed trimer comprising a gp41 fragment essential for trimer formation.

The specification also teaches at p. 9, ll. 28-30, "that the gp120 portion is sufficient to be recognized by neutralizing anti-gp120 antibodies and by CD4." Furthermore, a number of gp120 epitopes are known in the art. Thus, it would be no more than a routine matter to make and use the claimed compositions and methods directed to fragments of gp160 comprising gp41 and an immunogenic fragment of gp120.

In summary, the claims have been amended to recite not any fragment of gp160, but only specific fragments of the component gp41 and gp120 proteins that are known in the art to impart the desired properties.

Claims 11-19 were also rejected for lacking enablement. The Office Action alleged that the specification did not enable one of ordinary skill in the art to make the claimed constructs. The Office Action alleged that the prior art taught that following reduction of protein oligomers with a sulfhydryl-containing reducing agent, the reducing agent must be removed before blocking with N-ethylmaleimide. The Office Action further alleged that the example disclosed on p. 18, lines 1-20, did not teach removal of the sulfhydryl reducing agent before addition of N-ethylmaleimide and, therefore, one would not obtain the claimed trimers. Furthermore, the Office Action alleged that the specification failed to demonstrate that the claimed trimers were indeed obtained. For the following reasons, the applicant respectfully traverses.

The reaction of N-ethylmaleimide with proteins is well known and characterized in the art, dating back to before 1964 (as manifested by Smthy *et al.*, *Biochem J.* **91**, 589 (1964)). It is respectfully submitted that those of ordinary skill in the art would be just as cognizant as the Examiner regarding the conditions required for successful blocking of reduced sulfhydryl moieties on a protein when using a sulfhydryl reducing agent. Based on the well established and widely known use of N-ehtylmaleimide to block protein sulfhydryl groups, it would be no more than a routine matter for one of ordinary skill in the art to

determine the appropriate conditions and procedure for obtaining a trimer according to the invention by, *inter alia* reducing a multimer protein with a sulfhydryl containing reducing agent and blocking the resulting reduced protein. Furthermore, the Office Action has not provided any evidence that conducting the reactions in the manner the Examiner deems necessary would be beyond the skill level of the ordinary artisan.

Furthermore, contrary to the Office's assertion, the specification in fact provides experimental evidence that a trimer was obtained. The figure shows the results of an SDS PAGE analysis comparing gp160 trimers according to the invention (lanes 2 and 3) with gp160 monomers (lanes 5 and 6), dimers (lane 2), and a mixture of dimers, trimers, and tetramers (lane 7). As can be seen from the figure, the spots in lanes 2 and 3 migrate more slowly that the monomers of lanes 5-7 and the dimers of lanes 2 and 7, indicated that they contain a trimer.

Furthermore, attached hereto as Appendix A is a SDS-gel as requested in the Office Action. The gel clearly demonstrates that the trimers of the invention do no contain any inter-chain disulfide bonds. The applicants will provide the gel in the form of a Rule 132 declaration if deemed necessary.

The Office Action also complained that the order of the various actions in claims 18 and 19 was important, but not specified. The claims have been amended to obviate this issue.

The Office Action also complained with regard to claim 19 that the specification did not provide any indication that following the reduction and oxidation would lead to a trimer that does not contain inter-chain disulfide bonds, alleging that the ordinary artisan would expect that after oxidizing the previously reduced sulfur group the sulfur is then capable of forming disulfide bonds. The applicants respectfully submit that oxidation and reduction of proteins are well known, well characterized, and frequently used techniques by the ordinary artisan and that it would be merely a routine matter for one of ordinary skill in the art to determine the appropriate conditions in which to conduct reduction following by oxidation without the difficulties envisioned by the Office. Furthermore, the Office Action has not provided any evidence that conducting the reactions under the conditions the Examiner deems necessary would be beyond the skill level of the ordinary artisan.

Finally, with regard to the objection to the term "vaccine," the claims have been amended to remove the term because it is merely surplusage, being unnecessary to define the make-up of the claimed composition. The rejection is thereby obviated.

In view of all of the foregoing, the applicant respectfully submits that the claims are enabled.

If there are any questions or comments regarding this Response or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,

Date: February 6, 2003

Michael S. Greenfield
Registration No. 37,142

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, IL 60606

Telephone: 312-913-0001
Facsimile: 312-913-0002

New Claims and redlined version of amended claims

11. (Amended) A composition comprising a purified trimer of ~~a naturally-occurring or recombinant~~ HIV gp160, wherein the trimer:

a) binds to CD4;

b) binds to an anti-gp120 antibody capable of neutralizing HIV infection of cells *in vitro*;

c) binds to an anti-gp41 antibody; and

d) has no inter-chain disulfide bridges.

13. (Amended) ~~The~~ A composition ~~of claim 11,~~ comprising a trimer of HIV gp160 wherein all or a portion of the gp160 transmembrane region is deleted, and were the trimer:

e) binds to CD4;

f) binds to an anti-gp120 antibody capable of neutralizing HIV infection of cells *in vitro*;

g) binds to an anti-gp41 antibody; and

h) has no inter-chain disulfide bridges.

16. (Amended) ~~A vaccine comprising t~~The composition of any one of claims 11 - 13 ~~and~~ further comprising an adjuvant.

17. (Amended) ~~A vaccine~~ The composition according to claim 16 wherein the trimer is the only HIV surface antigen in the ~~vaccine~~composition.

18. (Amended) A method of producing the trimer according to any one of claims 11 - ~~13~~12, the method comprising, in order:

a) expressing gp160 ~~or fragment thereof~~;

b) purifying the gp160;

c) contacting the gp160 with a reducing agent;

d) contacting the gp160 with an alkylating agent;

e) contacting the gp160 with an oxidizing agent;

f) contacting the gp160 with an ionic detergent, and

g) dialyzing the gp160 against a neutral detergent.

19. (Amended) A method of producing the trimer according to any one of claims 11-~~13~~12, the method comprising, in order:

a) expressing gp160~~ or fragment thereof~~;

a) purifying the gp160;

b) contacting the gp160 with an ionic detergent;

c) contacting the gp160 with a reducing agent;

d) contacting the gp160 with an oxidizing agent; and

e) dialyzing the gp160 against a neutral detergent.

20. (New) A method of producing the trimer according to claim 13, the method comprising, in order:

a) expressing gp160 having its transmembrane region deleted therefrom;

b) purifying the gp160;

c) contacting the gp160 with a reducing agent;

d) contacting the gp160 with an alkylating agent;

e) contacting the gp160 with an oxidizing agent;

f) contacting the gp160 with an ionic detergent, and

g) dialyzing the gp160 against a neutral detergent.

21. (New) A method of producing the trimer according to any one of claims 13, the method comprising, in order:

a) expressing gp160 having its transmembrane region deleted therefrom;

b) purifying the gp160;

c) contacting the gp160 with an ionic detergent;

d) contacting the gp160 with a reducing agent;

e) contacting the gp160 with an oxidizing agent; and

f) dialyzing the gp160 against a neutral detergent.

22. (New) A composition comprising a purified trimer of HIV gp160 comprising a gp41 fragment essential for trimer formation and an immunogenic fragment of gp120, wherein the trimer:

i) binds to CD4;

j) binds to an anti-gp120 antibody capable of neutralizing HIV infection of cells *in vitro*;

k) binds to an anti-gp41 antibody; and

l) has no inter-chain disulfide bridges.

23. (New) The composition according to claim 22, wherein the gp41 and gp120 are from different HIV strains.

24. (New) The composition according to any one of claims 22 - 23 having a protein content that comprises more than 50% of the trimer.

25. (New) The composition according to any one of claims 22 - 23 wherein the binding affinity of the trimer to CD4 is equal or greater than the binding affinity of gp120 of an infectious HIV.

26. (New) The composition of any one of claims 22 - 23 further comprising an adjuvant.

27. (New) The composition according to claim 26 wherein the trimer is the only HIV surface antigen in the composition.

28. (New) A method of producing the trimer according to any one of claims 22 - 23, the method comprising, in order:

a) expressing a gp160 fragment comprising gp41 and an immunogenic gp120 fragment;

b) purifying the gp160 fragment;

c) contacting the gp160 fragment with a reducing agent;

d) contacting the gp160 fragment with an alkylating agent;

e) contacting the gp160 fragment with an oxidizing agent;

f) contacting the gp160 fragment with an ionic detergent, and

g) dialyzing the gp160 fragment against a neutral detergent.

29. (New) A method of producing the trimer according to any one of claims 22- 23, the method comprising, in order:

a) expressing a gp160 fragment comprising gp41 and an immunogenic gp120 fragment;

b) purifying the gp160 fragment;

c) contacting the gp160 fragment with an ionic detergent;

d) contacting the gp160 fragment with a reducing agent;

e) contacting the gp160 fragment with an oxidizing agent; and

f) dialyzing the gp160 fragment against a neutral detergent.

30. (New) The composition according to claim 22 wherein the gp41 fragment essential for trimer formation comprises gp41 lacking its transmembrane domain.

31. (New) The composition according to claim 30 wherein the gp41 fragment comprises the 129 N-terminal amino acids of gp41.